MICROSTIMULATION OF LUMBOSACRAL SPINAL CORD-MAPPING

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I. Introduction

During this quarter progress was made in three areas of investigation: (1) Mapping of sites in the lumbosacral spinal cord which produce changes in cavernous sinus pressure to focal microstimulation. (2) Tracing studies using the transsynaptic tracer, pseudorabies virus (PRV) to determine the location of efferent neurons and interneurons in the spinal cord which project to the extensor muscles of the lower hindlimb (shank). These muscles produce flexion and extension of the shank about the knee joint. (3) The mapping of sites in the lumbar spinal cord which produce flexion and extension of the shank to microstimulation of the L_6 spinal cord.

A brief description of the methods used in these studies and a summary of results are presented below.

II. Mapping of Sites in the Sacral Spinal Cord Which Produce Increases in Cavernous Sinus Pressure to Focal Microstimulation.

It is now well established experimentally that an increase in penile cavernous sinus pressure is correlated with penile erection in the cat, dog and rat and is inferred to be the mechanism of penile erection in other species including man.

In our studies we recorded cavernous sinus pressure in the pentobarbital (30mg/kg iv) anesthetized cat. A 22 gauge intracatheter is placed in the cavernous space following a small incision in the tip of the penis. The catheter is secured with a suture, similar to cannulation of a vein. In some experiments bladder pressure was also recorded via a catheter placed in the dome of the bladder. A laminectomy exposes the spinal cord and roots from L_6 to the S_3 spinal segments. The segment which produces the greatest rise in cavernous sinus pressure to ventral

root stimulation presumably contained the majority of efferent neurons to the penis. The S_1 ventral root produced the largest rise in cavernous sinus pressure in 10 of 11 cats tested while S_2 root stimulation was greatest in one animal. There was little or no response from either the L_7 or S_3 ventral roots in any animal tested. The S_2 root usually gave some response although smaller than S_1 in all but one animal mentioned above. This data suggests that the S_1 and S_2 spinal segment contains the majority of parasympathetic excitatory efferent neurons to the penis, while the L_7 and S_3 segments contained little or no efferent neurons to the penis. This conclusion is supported by our tracing studies as well.

The S_1 and S_2 segments of the cord were then mapped with fine tipped (300-400μ^2 exposed surface area) activated iridium microelectrodes. The electrodes were moved in 200μ increments along an electrode tract and each point was stimulated with negative first charge balanced pulses 200μsec duration, 25-35Hz, 25-150μA for 1 minute, every four minutes. A four-minute interval was used between stimulus presentation since it was noted that some long term (1-3min.) inhibition is produced if stimulus is repeated at intervals shorter than three minutes. A typical response to S_1 cord stimulation is shown in Figure 1. Both bladder and penile pressure (cavernous sinus pressure) are recorded in this experiment and shown in Figure 1. The bladder response has a rapid onset but a short duration of action and the peak pressure is just above 20 cm of H_2O. This type of bladder response is expected since neither the location of the stimulating microelectrode (in caudal S_1) or the stimulus parameters are ideal for bladder responses. The penile response is quite large, with a rather long onset (typically 8-20 secs) and a duration which often outlasts the stimulus. The peak pressure is quite high and approaches systolic blood pressure and is often similar to the response seen with the ventral root stimulation.

Changes in stimulus parameter will alter the peak amplitude, duration and the onset of the pressure response. Figure 2 shows the effect of changing the intensity of stimulation from 25μA, to 50μA, and 100μA, (Figure 2 A, B, & C). With decrease in stimulus intensity there is a decrease in amplitude and duration and a marked increase in latency of the pressure response.

Frequency is also an important stimulus parameter to maximize the penile pressure response. Figure 3 shows the effect of varying stimulation frequency on peak amplitude, duration, and area under the pressure response curve. The frequency which produces the largest cavernous sinus pressure response is between 25-35Hz. This frequency is in marked contrast to that which produces the maximal bladder response, usually 15-20 Hz.

The location in the spinal cord where microstimulation produces the largest cavernous sinus pressure changes are seen in the middle and caudal S_1 segment. Figure 4 (left) shows the distribution of responses in the S_1 cord which produce a greater than 30 cm H_2O rise in cavernous sinus pressure. The region with the lowest threshold for activation is a small area beginning in the dorsal-ventral horn near the sacral parasympathetic nucleus and extending to the base of the ventral horn. This area corresponds to an area of the ventral horn which contains a group of neurons which are labeled by pseudorabies virus following injection into the penis, Figure 4 (right). The labeled neurons are sacral parasympathetic neurons (SPN), interneurons, and some large motor neurons. The SPN neurons are known to innvervate the vasculature of the penis and are probably involved in penile erection as are the large motor neurons which likely innervate the ishocavernous and bulbocavernous striated muscles. Both these striated muscles are thought to be involved in sexual function. In fact the simultaneous activation of both the SPN neurons and the motor neurons often produce the largest pressure changes.

The mapping studies for penile pressure changes in intact cats are nearly complete and a manuscript is being prepared for publication and should be completed during the next quarter.

III. The Location and Distribution of Motoneurons and Interneurons Which Project to the Shank Extensor Muscles of the Cat Hindlimb.

Although some information is available in the literature (Romanes, 1951) concerning the location of hindlimb motor cell columns in the cat spinal cord, the present studies were designed to reexamine and extend these older studies using modern tracing techniques. Pseudorabies virus, a transsynaptic tracer, was used to label efferent neurons and interneurons in the lumbar spinal cord.

The Becker strain of pseudorabies virus was injected into the quadriceps muscles of "immunologically naive" cats. The quadriceps are the one group of extensors which extend the lower hindlimb about the knee joint. Two different methods of injection of PRV were tried; (1) The entire quadriceps femoris muscle of one leg was injected at multiple sites with PRV. Approximately 60 sites were injected with 5μl each of PRV. The quadriceps femoris is a large muscle which covers the anterior surface of the thigh and consists of four muscle bundles. Although the cats used in this study were small (1.5-2.0kg) and the volume of virus needed was quite large, the entire quadriceps were not completely infiltrated with virus. In an attempt to label most of the neurons which innervate this muscle the nerve bundle which projects to the quadriceps were identified in the opposite leg and in this experiment the nerve injected directly with 10μl PRV. Unfortunately the nerve injection produced no labeled neurons in the spinal cord while the muscle injection produced both labeled motor neurons and interneurons in the L_6

and L_7 spinal cord as well as the dorsal root ganglia. The labeled motoneurons were located deep at the base of the ventral horn, they were of large (60-80μ) diameter and had extensive processes which could be followed in adjacent tissue sections. The interneurons were located along the lateral edge and more dorsal in the ventral horn. These neurons were much smaller (10-20μ in diameter) and had fewer processes. The processes that were present projected medially toward the central canal and ventrally toward the base of the ventral horn. Because of the distinct morphology and their location, compared with what is known for motor neurons these labeled neurons were considered to be interneurons. The number of neurons labeled in these experiments were fewer than that observed with organs such as the bladder and penis, which are innervated primarily by the autonomic nervous system. This is consistent with reports in the rat that motor systems label less densely than autonomic or sensory neurons with pseudorabies virus.

These studies well continue into the next quarter.

IV. Mapping of Sites in the Lumbosacral Spinal Cord Which Produce Flexion and Extension of the Lower Hindlimb to Microstimulation.

The purpose of this study is to determine the location of sites in the spinal cord which when activated by focal electrical stimulation using fine tipped microelectrodes would produce selectively, flexion or extension of the shank. It is more important in these studies to accurately determine the location, and stimulus parameters than to generate maximal flexor or extensor torque.

The details of the methods used have been presented in a previous progress report and

will only be briefly described here. Cats are anesthetized with halothane:oxygen anesthesia during initial surgical procedures. All recordings of motor responses from the hindlimb are done using pentobarbital anesthesia (30mg/kg iv, supplemented every few hours as necessary). A bracket attached to the tibia with two screws and clamped to the shaft of a rotational torque transducer is used to measure the isometric torque generated by the flexors and extensors of the shank. A laminectomy exposes the spinal cord and roots. The dura is cut and the surface of the spinal cord is superfused with warm oxygenated Krebs solution. Fine tipped (300-400μ^2 exposed surface area) activated iridium microelectrodes are used for mapping. The stimulus parameters for these motor studies are 200μsec duration negative first, biphasic balanced pulses, 35-40Hz, 25-100μA delivered for 30 seconds every 2 minutes. The frequencies of 35-40 Hz, determined in our preliminary study, are important in producing fused responses of maximal amplitude and with minimal amount of fatigue. The electrode is advanced in 200μ steps beginning at the cord surface and the spinal cord stimulated at one or two intensities at each site. Each adjacent penetration through the cord is at 200-300μ increments.

Figure 5 (top) shows a typical motor response to stimulation of the L_6 spinal cord at a depth of 2.6mm from the surface of the cord. This site produced an extensor response and is shown as a downward or negative deflection. The response had a rapid onset and return to control level as soon as the stimulation was stopped. The extension remained quite constant throughout the stimulation. The stimulus parameters for this response (Figure 5, top) 40 Hz at 100μA. At the same site at stimulus frequency of 80 Hz (Figure 5, bottom) produced a slightly larger initial response followed by fatigue of the extension over the next 30 sec. to a level less than one-half the initial response. By doing a number of frequency response curves in different

animals it was determined that a frequency of 35-40 Hz gave the largest response and the smallest degree of fatigue. It should be pointed out that the extension response is a net torque measure and could be a summation of extension and flexion occurring simultaneously. Although there was no obvious contraction of the flexor muscles this possibility will be investigated further with simultaneous EMG recording in these experiments.

The variation in response characteristics seen at different sites in the L_6 spinal cord are shown in Figure 6, A-D. Medial sites as well as much of the dorsal horn and dorsal root entry zone produce mostly flexion torque, of a low amplitude. This probably represents reflexes due to activation of afferent fibers or interneurons in the dorsal horn and the dorsal aspect of the ventral horn. Sites deep in the ventral horn produce mainly extension which is often large in magnitude especially when the microelectrode tip is located near the base of the ventral horn.

The extensor responses seen in the L_6 cord were also present in caudal L_5 and rostral L_7. The motor responses form S_1 and especially L_7, although not studied extensively, appeared complex and often involved foot, ankle, and toe movements as well as flexion and extension of the shank. This is to be expected since the L_7 motor columns are more diverse and L_7 ventral root stimulation produce responses in many parts of the leg and foot.

These studies will be continued into the next quarter and will include acute and chronic spinal cord transected animals, and more detail studies of S_1, L_7, and L_5.

Romanes, G.J. (1951). The motor cell columns of the lumbosacral spinal cord of the cat. *Journal of Comparative Neurology*, **94**, 313-363.

Figure 1. A plot of pressure versus time showing the change in penile pressure and bladder pressure to microstimulation of the S_1 spinal cord with the electrode tip 2.0mm below the cord surface. Arrows indicated the beginning and end of a 30 sec. stimulation. Notice the small amplitude, short duration bladder response during stimulation. Penile response is large with a long duration (outlasting the stimulation) and delayed onset of nearly 10secs. Stimulus parameters are: 200μsec pulses at 30 Hz, 100μA, 30 secs. "on" and 120 secs. "off."

Figure 2. A plot of penile pressure versus time showing the change in penile pressure to microstimulation of the S_1 spinal cord at three different intensities of stimulation. A. 100μA, B. 50μA, and C. 25μA. The arrows mark the duration of a 30 sec. stimulation. The tip of the electrode is 2.2 mm below the cord surface located in the middle of the ventral horn. Notice the increase in amplitude and duration and the decrease in latency of the penile response with increases in intensity of stimulation. Stimulus parameters are: 200 μsec. pulses at 30 Hz, 100μA (A), 50μA (B), and 25μA (C), 30sec. "on" 120sec. "off."

Figure 3. A plot showing the effects of stimulus frequency on percent maximum amplitude, duration, and area under the cavernous sinus pressure curve to S_1 spinal cord microstimulation for mean of four animals. Notice that the response at 20 Hz and below are very small but increased with increasing frequency. At 45 Hz and higher the response slowly decreases in size.

Figure 4. A diagram of two transverse sections of S_1 spinal cord showing (left) the distribution of microstimulation sites which produce a greater than 30 cm H_2O in cavernous sinus pressure (CSP) and (right) the distribution of pseudorabies virus (PRV) labeled neurons following penile injection of PRV. Notice the overlap of labeled neurons with active sites which produce large CSP changes.

Figure 5. A plot of flexor and extensor torque generated at the knee joint versus time showing the effects of L_6 cord microstimulation at two stimulus frequencies 40 Hz (top plot) and 80 Hz (bottom plot). Stimulus last 30 sec. and begins at 5 sec. and ends at 35 sec. for both plots. Extension is a downward movement of the curve while flexion is upward. This is a typical plot of the extensor response seen with L_6 cord simulation. Notice the fatigue which occurs with increased frequency. Our mapping studies are done at 35-45 Hz where fatigue is minimal. Stimulus parameters are: 200 μsec pulses at 40 Hz (top plot) 80 Hz (bottom plot), 100 μA, 30 sec. "on" and 120 sec. "off."

Figure 6. Plots of maximum torque developed at the shank by microstimulation of the L_6 cord at different depths along four electrode tracts A, B, C, and D. The four plots are the maximum responses seen along the four electrode tracts shown on the transverse L_6 spinal cord diagram at the center of the figure. Each tic on the electrode tracts represent a 0.2 mm movement of the electrode and corresponds to responses seen on each graph. Flexion is an upward and extension a downward movement of the curve in each graph. Torque is measured in newton-centimeters (Ncm). Notice that the stimulus sties which are dorsal and medial in the ventral horn or lateral in the white matter of the lateral funiculus produce mostly weak flexion while those sites deep in

the ventral horn produces often quite strong extension. Stimulus parameters for all sites are the same: 100 μA, 200 μsec pulses, at 40 Hz, for 30 secs. "on" and 120 sec. "off."

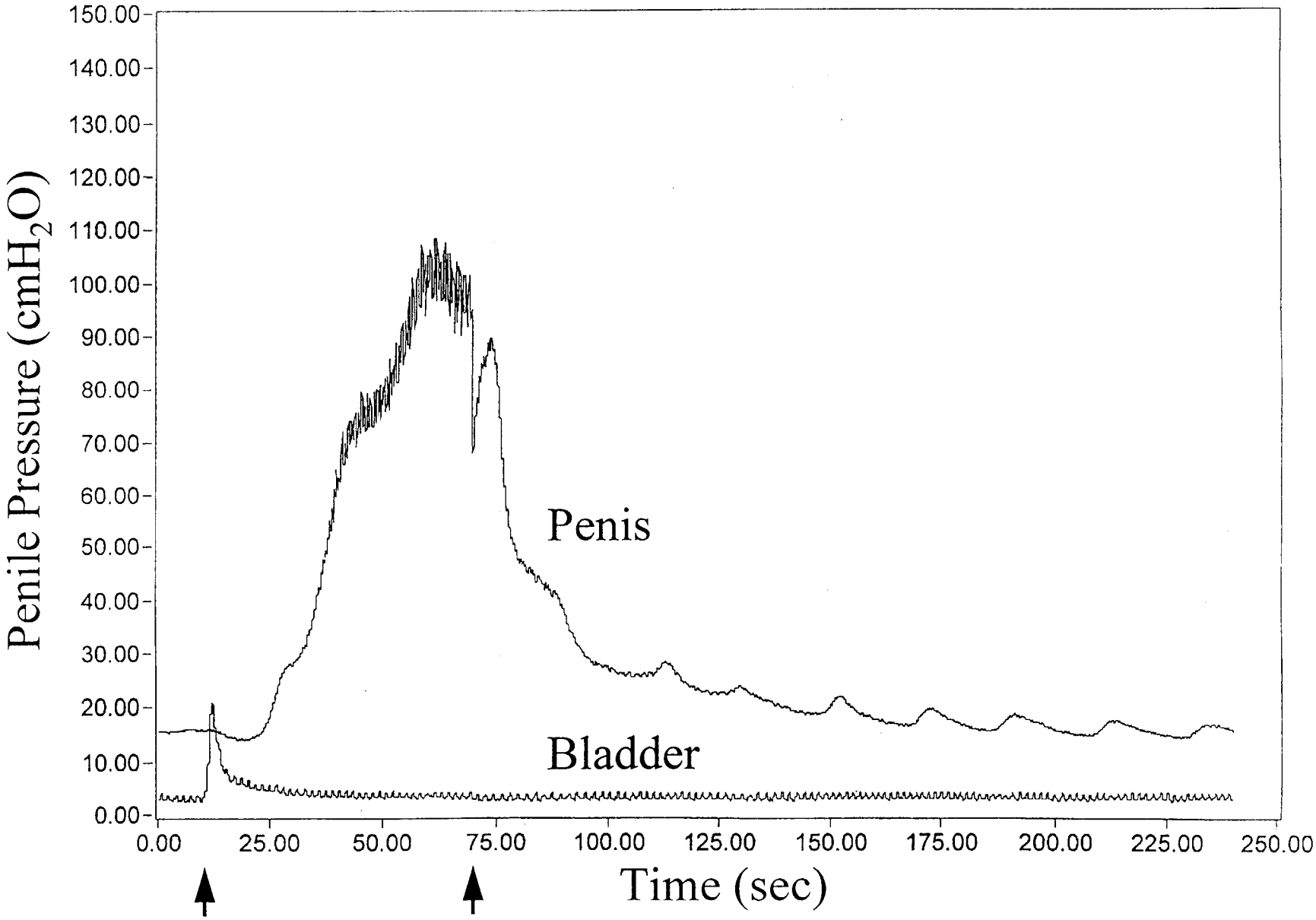

Penile Pressure (cmH_2O)
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Penis
Bladder
0.00
25.00
50.00
75.00
100.00
125.00
150.00
175.00
200.00
225.00
250.00
Time (sec)

Figure 1.

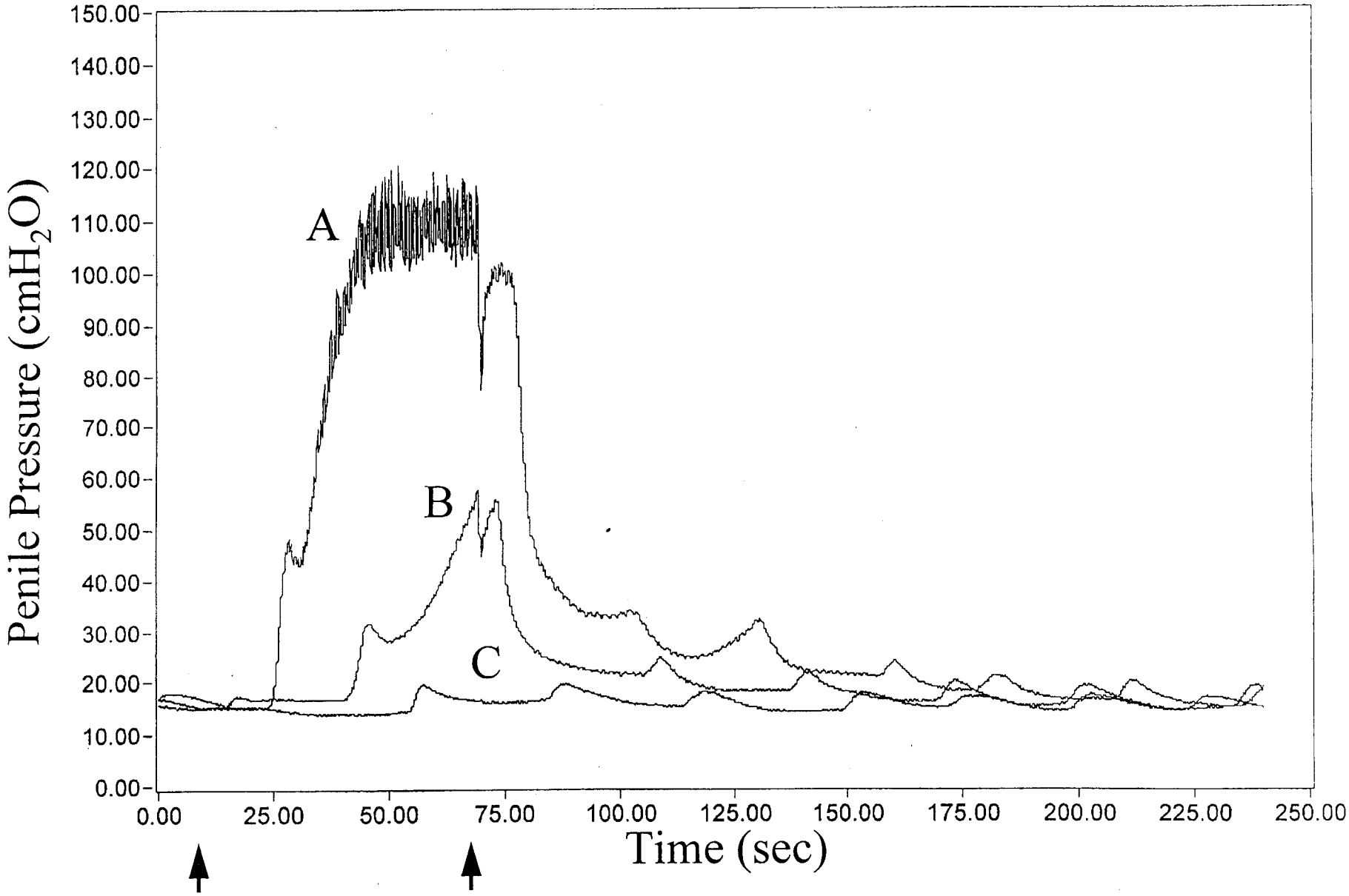

Penile Pressure (cmH2O)
150.00
140.00
130.00
120.00
110.00
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
A
B
C
0.00
25.00
50.00
75.00
100.00
125.00
150.00
175.00
200.00
225.00
250.00
Time (sec)

Figure 2.

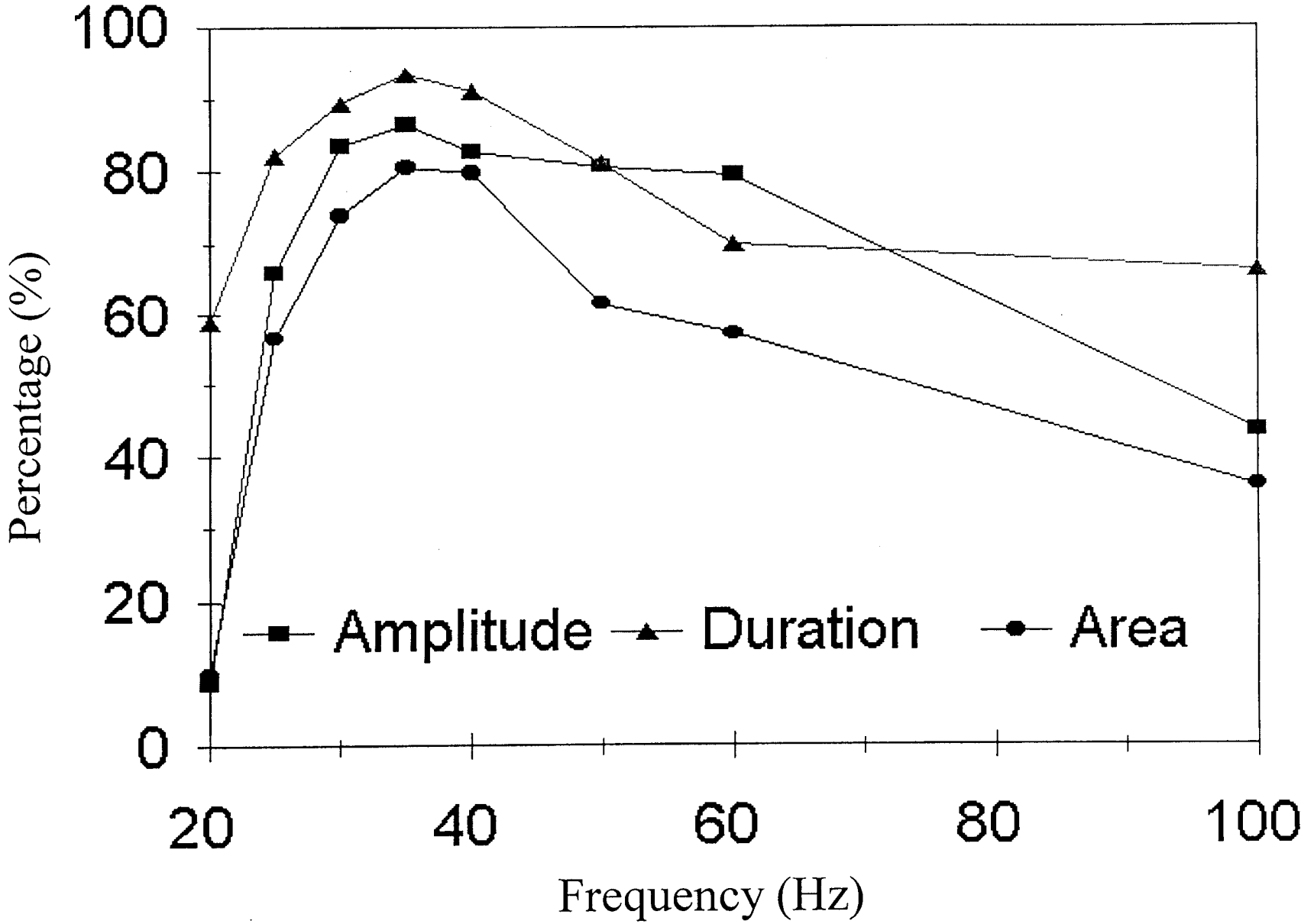
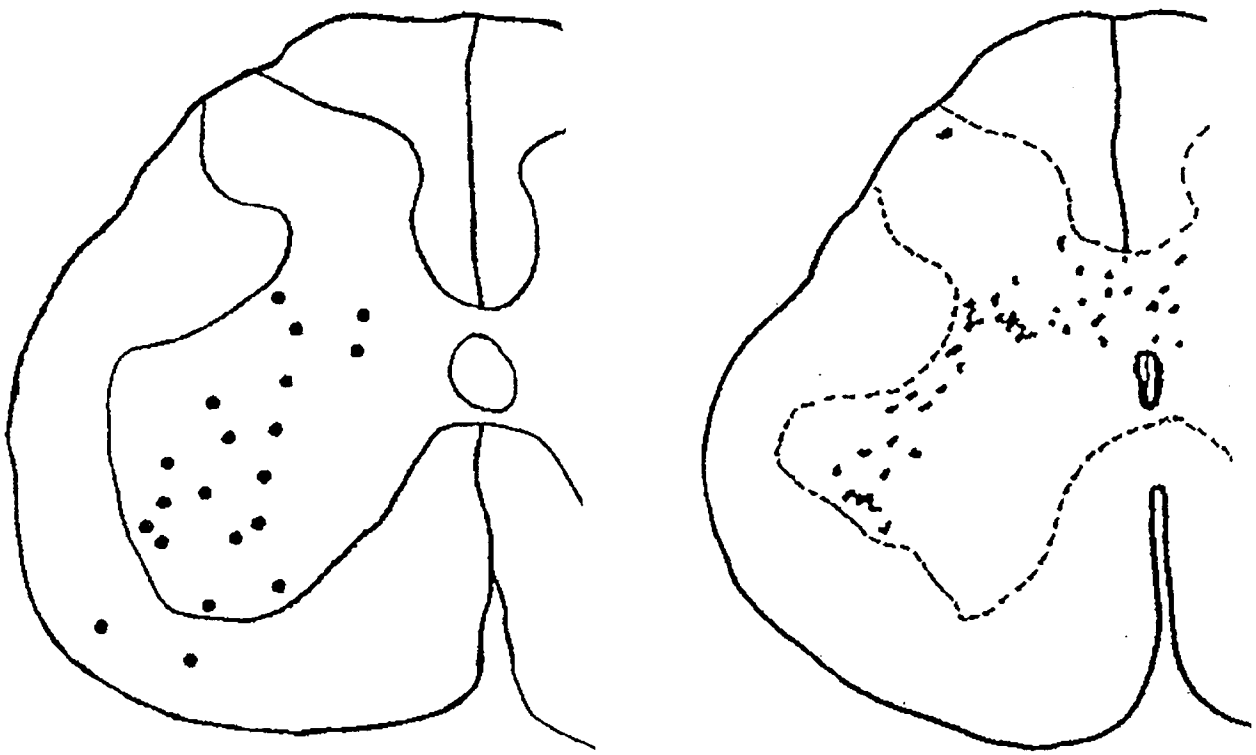

100
80
60
40
20
0
Percentage (%)
Amplitude
Duration
Area
20
40
60
80
100
Frequency (Hz)

Figure 3.

S1 Spinal Cord

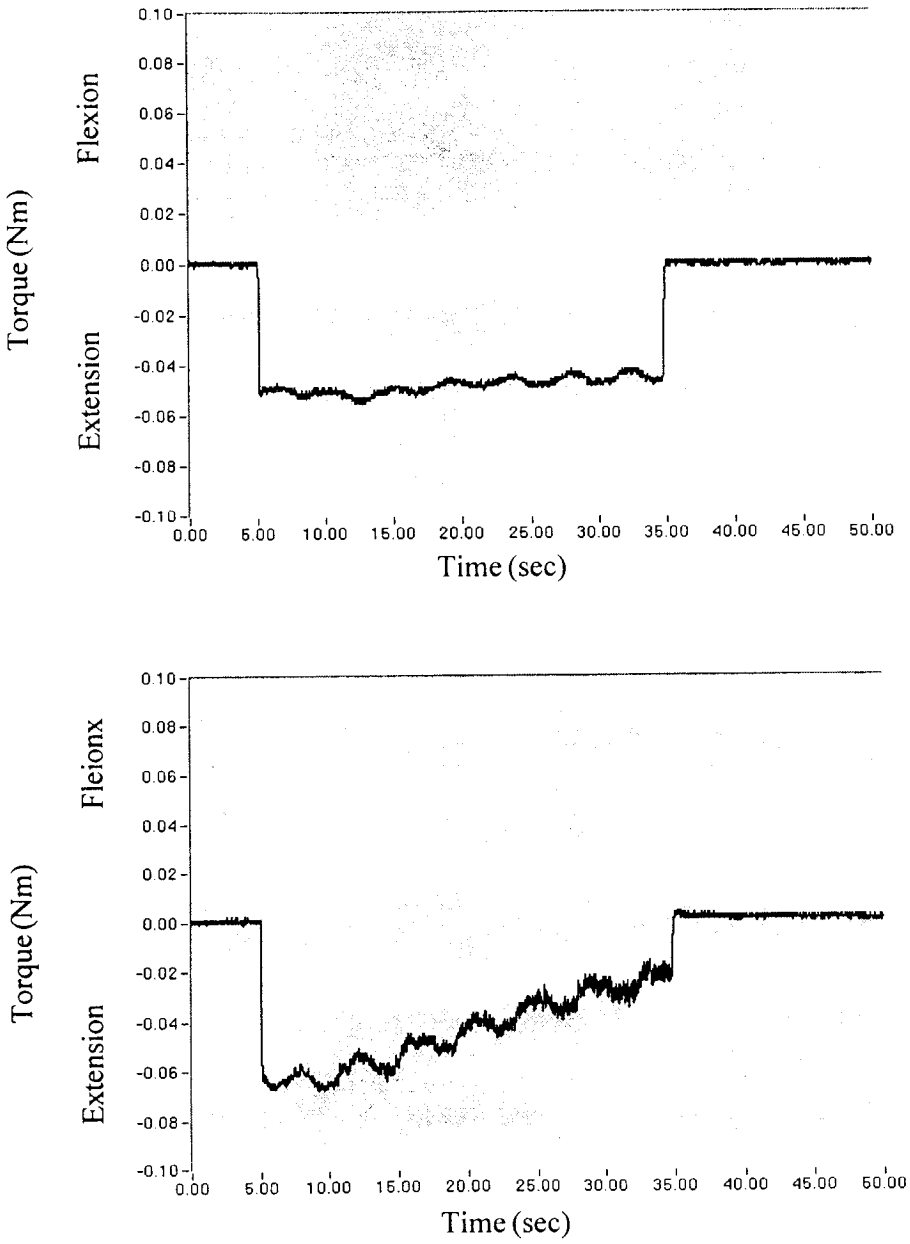


Microstimulation Sites
CSP>30cmH_2O

PRV Labeled Neurons
Following Penile Injection

1mm

Figure 4.


Torque (Nm)
Flexion
Extension
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)
Torque (Nm)
Fleionx
Extension
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
0.00
5.00
10.00
15.00
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25.00
30.00
35.00
40.00
45.00
50.00
Time (sec)

Figure 5.

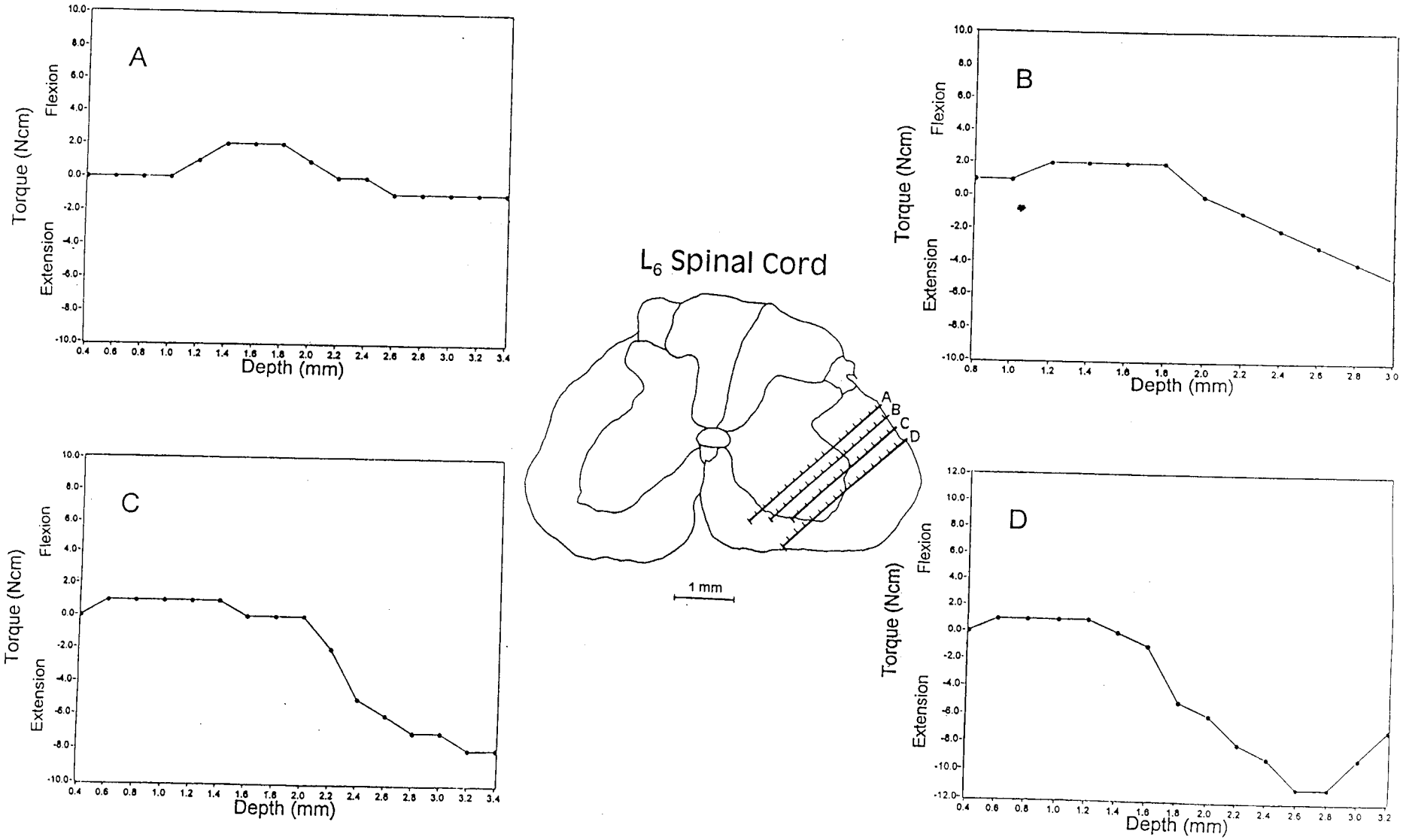
A
B
C
D
Torque (Ncm)
Flexion
Extension
Depth (mm)
L6 Spinal Cord
1 mm

Figure 6.